Bulletin of Faculty of Pharmacy, Cairo University (2015) 53, 37–43

HOSTED BY

Cairo University

Bulletin of Faculty of Pharmacy, Cairo University

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www.sciencedirect.com

ORIGINAL ARTICLE

Synthesis of some new 5-substituted-2-((6-chloro-3,4-methylenedioxyphenyl)methylthio)-1,3,4-oxadiazole derivatives as suitable antibacterial inhibitors

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Received 2 June 2014; accepted 21 October 2014
Available online 12 November 2014

KEYWORDS

1,3,4-Oxadiazoles;
Antibacterial activity;
Carboxylic acids;
Spectral analysis

Abstract Heterocyclic molecules belong to the most attractive group owing to their broad spectrum of antimicrobial activities. In the undertaken research, a number of new 5-substituted-2-((6-chloro-3,4-methylenedioxyphenyl)methylthio)-1,3,4-Oxadiazole derivatives (**6a–l**) were synthesized by converting various aryl/aralkyl carboxylic acids (**1a–l**) into corresponding esters (**2a–l**), carbohydrazides (3**a–l**) and 5-substituted-1,3,4-Oxadiazol-2-thiols (**4a–l**). The last step included the synthesis of target molecules, **6a–l**, by stirring **4a–l** and 6-chloro-3,4-methylenedioxybenzyl chloride (**5**) in a polar aprotic solvent. The structures of all the synthesized molecules were corroborated through spectral analysis. The screening of these molecules against antibacterial activity rendered them moderate inhibitors and most likely against *Escherichia coli*, relative to the reference standard, ciprofloxacin.

1. Introduction

Heterocyclic molecules such as Oxadiazoles have been synthesized and evaluated for medical and agricultural activities. The disubstituted Oxadiazoles have executed a range of pharmacologic activities. 1,3,4-Oxadiazole heterocycle has displayed many activities like antibacterial, antifungal, hypoglycemic and anti-inflammatory. It also possesses an important place in medicinal chemistry. Chemists are much interested in 2,5-disubstituted 1,3,4-Oxadiazoles because of their antimicrobial activities.[1–6] The heterocycle, 3,4-methylenedioxyphenyl ring is also the part of active drugs. The molecules employed as antitumor and anticancer possessing this ring are lycoricidine, narciclasine and pancratistatin.[7] Moreover, the antidepressant drugs such as paroxetine also bear this moiety.[8]

The structural modification leads to variation in antimicrobial activities of the molecules. This prompted us to extend our previous work[9–11] to synthesize 5-substituted-2-((6-chloro-3,

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Peer review under responsibility of Faculty of Pharmacy, Cairo University.

http://dx.doi.org/10.1016/j.bfopcu.2014.10.001
1110-0931

4-methylenedioxyphenyl)methylthio)-1,3,4-Oxadiazole derivatives with an aim to observe their antibacterial activity. The synthesized molecules were found to be better inhibitors of *Escherichia coli* and *Pseudomonas aeroginosa* relative to ciprofloxacin.

2. Materials and methods

2.1. General

The reagents, purchased from Alfa Aesar and Sigma–Aldrich, were of synthetic grade and the solvents, obtained through commercial suppliers, were of analytical grade. Melting points of the synthesized molecules were measured on Griffin-George apparatus in an open capillary tube and were uncorrected. Purity and reaction progress were monitored through thin layer chromatography (TLC) on coated silica gel G-25-UV_{254} plates using different ratios of ethyl acetate and *n*-hexane as solvent systems. KBr (potassium bromide) pellet procedure was used to record I.R. spectra on a Jasco-320-A spectrophotometer. Wave number is given in cm^{-1}. Bruker spectrometers are employed for ^{1}H NMR & ^{13}C NMR spectra in $CDCl_3$ at 400–100 MHz. Chemical shifts are given in ppm relative to TMS and coupling constant in Hz. EIMS were recorded through JMS-HX-110 spectrometer, with a data system.

2.2. General procedure for the synthesis of esters (**2a–l**)

The aryl/aralkyl carboxylic acids (**1a–l**; 2.0 g) were dissolved in 8.0 mL absolute ethanol followed by the addition of 1.0 mL concentrated sulfuric acid in a 100 mL round bottom (RB) flask. The mixture was refluxed for 2–5 h. After reaction completion, established by TLC (ethyl acetate:*n*-hexane, 20%:80%), the mixture was poured into a 250 mL separating funnel. Then 80 mL distilled water and concentrated aqueous sodium carbonate solution were added to set a pH [9,10]. The solvent, 30 mL diethyl ether was added and upper ether layer containing required ester was collected after shaking. The solvent was distilled off to afford the transparent esters, **2a–l**, with yields ranging 62–76%.[9–10]

2.3. General procedure for the synthesis of hydrazides (**3a–l**)

The esters (**2a–l**; 0.02 mol) were added to 20.0 mL ethanol in a 100 mL RB flask followed by 4.0 mL 80% hydrazine hydrate. The mixture was stirred or refluxed accordingly for 3–6 h till reaction completion, supervised by TLC (ethyl acetate:*n*-hexane, 40%:60%). The precipitates of solid products were generated after addition of excess of distilled water which were filtered and washed with distilled water to afford **3a–l**, with yields ranging 71–87%.[9,10]

2.4. General procedure for the synthesis of 5-substituted-1,3,4-Oxadiazol-2-thiols (**4a–l**)

The carbohydrazides (**3a–l**; 0.02 mol) were suspended in 20.0 mL absolute ethanol in a 100 mL RB flask, basified with potassium hydroxide (0.02 mol) and refluxed to homogenize the mixture. Carbon disulfide (0.04 mol) was poured to the mixture and refluxed it for 4–6 h. TLC (ethyl acetate:*n*-hexane, 30%:70%) was developed to verify the reaction completion. The mixture was diluted by 30–50 mL distilled water and dilute HCl was added to acidify up to pH = 3–4. The precipitates, **4a–l**, were collected through filtration and washed by distilled water. The compounds were obtained with yields ranging 74–81%.[9,10]

2.5. General procedure for the synthesis of 5-substituted-2-((6-chloro-3,4-methylenedioxyphenyl)methylthio)-1,3,4-Oxadiazole (**6a–l**)

5-substituted-1,3,4-Oxadiazol-2-thiols (**4a–l**; 0.2 mmol) were homogeneously dissolved in 15.0 mL *N,N*-dimethylformamide (DMF) in a 50 mL RB flask. After activation of **4a–l** by sodium hydride (0.2 mmol) on stirring for half an hour, 6-chloro-3,4-methylenedioxybenzyl chloride (**5**; 0.2 mmol) was added and further stirred for 3–5 h. After complete reaction, monitored through TLC (ethyl acetate:*n*-hexane, 30%:70%), cold distilled water was added and the products were isolated by filtration or solvent extraction. The final solid products were re-crystallized from methanol.

2.5.1. 5-Phenyl-2-((6-chloro-3,4-methylenedioxyphenyl)methylthio)-1,3,4-Oxadiazole (**6a**)

White amorphous solid; Yield: 99%; M.P: 155 °C; HR-MS: $[M]^+$ 346.0176 (Calcd. for $C_{16}H_{11}ClN_2O_3S$; 346.0183); IR (KBr): ν_{max} (cm^{-1}): 3053 (Ar—H stretching), 1533 (Ar C=C stretching), 1125 (C—O stretching), 711 (C—Cl stretching), 610 (C—S stretching); ^{1}H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.98 (dd, J = 8.0, 1.2 Hz, 2H, H-2″ & 6″), 7.50–7.45 (m, 3H, H-3″ to 5″), 7.09 (s, 1H, H-2′), 6.84 (s, 1H, H-5′), 5.94 (s, 2H, H-7′), 4.53 (s, 2H, H-8′); ^{13}C NMR ($CDCl_3$, 100 MHz, δ/ppm): 165.4 (C-5), 164.6 (C-2), 150.3 (C-4′), 148.7 (C-3′), 135.2 (C-3″ & 5″), 132.8 (C-2″ & 6″), 130.4 (C-4″), 129.8 (C-1″), 129.5 (C-1′), 125.7 (C-6′), 110.9 (C-5′), 110.2 (C-2′), 101.9 (C-7′), 32.7 (C-8′); EIMS (m/z): 348 $[M+2]^+$ (0.3%), 346 $[M]^+$ (1%), 311 (100%), 200 (2%), 178 (2%), 169 (8%), 145 (12%), 139 (4%), 134 (3%), 119 (7%), 105 (8%), 104 (4%), 103 (5%), 77 (9%), 51 (3%).

2.5.2. 5-(2-Methylphenyl)-2-((6-chloro-3,4-methylenedioxyphenyl)methylthio)-1,3,4-Oxadiazole (**6b**)

White amorphous solid; Yield: 90%; M.P: 109 °C; HR-MS: $[M]^+$ 360.0332 (Calcd. for $C_{17}H_{13}ClN_2O_3S$; 360.0341); IR (KBr): ν_{max} (cm^{-1}): 3058 (Ar—H stretching), 1539 (Ar C=C stretching), 1121 (C—O stretching), 707 (C—Cl stretching), 617 (C—S stretching); ^{1}H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.84 (d, J = 7.6 Hz, 1H, H-6″), 7.40 (t, J = 7.2 Hz,1H, H-4″), 7.32 (d, J = 7.6 Hz, 1H, H-3″), 7.28 (t, J = 7.6 Hz, 1H, H-5″), 7.12 (s, 1H, H-2′), 6.85 (s, 1H, H-5′), 5.95 (s, 2H, H-7′), 4.53 (s, 2H, H-8′), 2.67 (s, 3H, H-7″); ^{13}C NMR ($CDCl_3$, 100 MHz, δ/ppm): 164.8 (C-5), 164.1 (C-2), 150.1 (C-4′), 149.4 (C-3′), 134.3 (C-2″), 131.6 (C-1′), 130.9 (C-4″), 128.8 (C-3″), 128.1 (C-1″), 127.6 (C-5″), 126.8 (C-6′), 126.2 (C-6″), 111.2 (C-5′), 110.6 (C-2′), 101.8 (C-7′), 32.5 (C-8′), 20.1 (C-7″); EIMS (m/z): 362 $[M+2]^+$ (0.5%), 360 $[M]^+$ (1.5%), 325 (100%), 200 (2%), 192 (5%), 169 (8%), 160 (2%), 139 (8%), 134 (3%), 133 (6%), 119 (4%), 117 (3%), 104 (9%), 91 (30%), 51 (2%).

*2.5.3. 5-(4-Methylphenyl)-2-((6-chloro-3,4-methylenedioxy phenyl)methylthio)-1,3,4-Oxadiazole (**6c**)*

White amorphous solid; Yield: 96%; M.P: 198 °C; HR-MS: $[M]^+$ 360.0332 (Calcd. for $C_{17}H_{13}ClN_2O_3S$; 360.0341); IR (KBr): ν_{max} (cm^{-1}): 3047 (Ar—H stretching), 1536 (Ar C═C stretching), 1118 (C—O stretching), 705 (C—Cl stretching), 617 (C—S stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.86 (d, J = 8.0 Hz, 2H, H-2″ & 6″), 7.20 (d, J = 8.4 Hz, 2H, H-3″ & 5″), 7.09 (s, 1H, H-2′), 6.84 (s, 1H, H-5′), 5.94 (s, 2H, H-7′), 4.52 (s, 2H, H-8′), 2.40 (s, 3H, H-7″); ^{13}C NMR ($CDCl_3$, 100 MHz, δ/ppm): 165.7 (C-5), 164.4 (C-2), 149.8 (C-4′), 149.2 (C-3′), 140.4 (C-4″), 130.9 (C-1′), 130.2 (C-2″ & 6″), 129.7 (C-1″), 129.1 (C-3″ & 5″), 125.6 (C-6′), 110.5 (C-5′), 110.0 (C-2′), 101.6 (C-7′), 32.9 (C-8′), 20.9 (C-7″); EIMS (m/z): 362 $[M+2]^+$ (0.3%), 360 $[M]^+$ (1%), 325 (100%), 200 (3%), 192 (4%), 169 (7%), 160 (3%), 139 (6%), 134 (4%), 133 (5%), 119 (6%), 117 (2%), 104 (7%), 91 (23%), 51 (6%).

*2.5.4. 5-(2-Nitrophenyl)-2-((6-chloro-3,4-methylenedioxy phenyl)methylthio)-1,3,4-Oxadiazole (**6d**)*

White amorphous solid; Yield: 97%; M.P: 125 °C; HR-MS: $[M]^+$ 391.0031 (Calcd. For $C_{16}H_{10}ClN_3O_5S$; 391.0037); IR (KBr): ν_{max} (cm^{-1}): 3061 (Ar—H stretching), 1542 (Ar C═C stretching), 1258 (C—N stretching), 1133 (C—O stretching), 710 (C—Cl stretching), 619 (C—S stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 8.01 (dd, J = 9.2, 1.6 Hz, 1H, H-3″), 7.92 (dd, J = 8.8, 1.6 Hz, 1H, H-6″), 7.74 (dt, J = 9.2, 2.0 Hz,1H, H-4″), 6.74 (t, J = 7.6 Hz, 1H, H-5″), 7.02 (s, 1H, H-2′), 6.85 (s, 1H, H-5′), 5.96 (s, 2H, H-7′), 4.52 (s, 2H, H-8′); ^{13}C NMR ($CDCl_3$, 100 MHz, δ/ppm): 165.1 (C-2), 164.2 (C-5), 150.4 (C-4′), 149.7 (C-3′), 146.2 (C-2″), 132.5 (C-4″), 130.8 (C-5″), 130.1 (C-1′), 127.9 (C-3″), 126.8 (C-6′), 126.2 (C-6″), 124.7 (C-1″), 110.9 (C-5′), 110.3 (C-2′), 101.5 (C-7′), 31.8 (C-8′); EIMS (m/z): 393 $[M+2]^+$ (0.3%), 391 $[M]^+$ (1%), 356 (25%), 222 (100%), 200 (4%), 192 (7%), 190 (5%), 169 (9%), 164 (90%), 150 (2%), 148 (5%), 139 (2%), 134 (3%), 122 (7%), 104 (3%), 51 (3%).

*2.5.5. 5-(3-Nitrophenyl)-2-((6-chloro-3,4-methylenedioxy phenyl)methylthio)-1,3,4-Oxadiazole (**6e**)*

Creamy white amorphous solid; Yield: 99%; M.P: 200 °C; HR-MS: $[M]^+$ 391.0031 (Calcd. For $C_{16}H_{10}ClN_3O_5S$; 391.0037); IR (KBr): ν_{max} (cm^{-1}): 3065 (Ar—H stretching), 1543 (Ar C═C stretching), 1253 (C—N stretching), 1134 (C—O stretching), 708 (C—Cl stretching), 616 (C—S stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 8.35 (d, J = 8.0 Hz, 1H, H-4″), 8.17 (d, J = 7.6 Hz, 1H, H-6″), 7.99 (s, 1H, H-2″), 7.92–7.90 (m,1H, H-5″), 7.13 (s, 1H, H-2′), 7.02 (s, 1H, H-5′), 5.95 (s, 2H, H-7′), 4.58 (s, 2H, H-8′); ^{13}C NMR ($CDCl_3$, 100 MHz, δ/ppm): 165.4 (C-5), 164.6 (C-2), 150.7 (C-4′), 150.0 (C-3′), 149.2 (C-3″), 136.3 (C-6″), 132.5 (C-1″), 131.2 (C-5″), 129.8 (C-1′), 126.3 (C-6′), 125.1 (C-4″), 122.6 (C-2″), 110.8 (C-5′), 110.0 (C-2′), 101.1 (C-7′), 32.4 (C-8′); EIMS (m/z): 393 $[M+2]^+$ (0.3%), 391 $[M]^+$ (1%), 356 (25%), 222 (100%), 200 (4%), 192 (7%), 190 (5%), 169 (9%), 164 (90%), 150 (2%), 148 (5%), 139 (2%), 134 (3%), 122 (7%), 104 (3%), 51 (3%).

*2.5.6. 5-(2-Chlorophenyl)-2-((6-chloro-3,4-methylenedioxy phenyl)methylthio)-1,3,4-Oxadiazole (**6f**)*

White amorphous solid; Yield: 98%; M.P: 160 °C; HR-MS: $[M]^+$ 379.9784 (Calcd. For $C_{16}H_{10}Cl_2N_2O_3S$; 379.9793); IR (KBr): ν_{max} (cm^{-1}): 3055 (Ar—H stretching), 1536 (Ar C═C stretching), 1127 (C—O stretching), 713 (C—Cl stretching), 614 (C—S stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.91 (dd, J = 8.0, 1.6 Hz, 1H, H-3″), 7.53 (dd, J = 8.0, 1.6 Hz, 1H, H-6″), 7.45 (dt, J = 7.6, 1.2 Hz, 1H, H-4″), 7.38 (dt, J = 7.6, 1.6 Hz, 1H, H-5″), 7.11 (s, 1H, H-2′), 6.85 (s, 1H, H-5′), 5.95 (s, 2H, H-7′), 4.55 (s, 2H, H-8′); ^{13}C NMR ($CDCl_3$, 100 MHz, δ/ppm): 167.3 (C-5), 165.3 (C-2), 150.7 (C-4′), 149.9 (C-3′), 132.8 (C-4″), 131.1 (C-2″), 130.7 (C-1′), 128.4 (C-3″), 126.7 (C-6″), 125.7 (C-5″), 124.3 (C-1″), 123.5 (C-6′), 110.8 (C-5′), 110.4 (C-2′), 101.4 (C-7′), 31.9 (C-8′); EIMS (m/z): 384 $[M+4]^+$ (0.3%), 382 $[M+2]^+$ (0.7%), 380 $[M]^+$ (1%), 345 (100%), 212 (3%), 200 (2%), 179 (16%), 169 (9%), 153 (85%), 139 (6%), 137 (5%), 134 (2%), 111 (8%), 104 (6%), 51 (3%).

*2.5.7. 5-(4-Hydroxyphenyl)-2-((6-chloro-3,4-methylenedioxy phenyl)methylthio)-1,3,4-Oxadiazole (**6g**)*

White amorphous solid; Yield: 98%; M.P: 145 °C; HR-MS: $[M]^+$ 362.0126 (Calcd. For $C_{16}H_{11}ClN_2O_4S$; 362.0136); IR (KBr): ν_{max} (cm^{-1}): 3216 (O—H stretching), 3060 (Ar—H stretching), 1537 (Ar C—C stretching), 1119 (C—O stretching), 703 (C—Cl stretching), 618 (C—S stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.88 (d, J = 8.4 Hz, 2H, H-2″ & 6″), 7.12 (s, 1H, H-2′), 6.91 (d, J = 8.8 Hz, 2H, H-3″ & 5″), 6.84 (s, 1H, H-5′), 5.94 (s, 2H, H-7′), 4.51 (s, 2H, H-8′); ^{13}C NMR ($CDCl_3$, 100 MHz, δ/ppm): 164.9 (C-5), 164.1 (C-2), 159.7 (C-4″), 150.0 (C-4′), 148.6 (C-3′), 130.6 (C-1′), 125.3 (C-6′), 124.4 (C-1″), 123.2 (C-2″ & 6″), 119.1 (C-3″ & 5″), 110.8 (C-5′), 110.1 (C-2′), 101.5 (C-7′), 32.7 (C-8′); EIMS (m/z): 364 $[M+2]^+$ (0.3%), 362 $[M]^+$ (1.5%), 327 (100%), 200 (2%), 194 (5%), 169 (9%), 161 (2%), 139 (4%), 135 (9%), 134 (20%), 121 (8%), 119 (6%), 104 (7%), 93 (15%), 51 (4%).

*2.5.8. 5-(4-Methoxyphenyl)-2-((6-chloro-3,4-methylenedioxy phenyl)methylthio)-1,3,4-Oxadiazole (**6h**)*

White amorphous solid; Yield: 98%; M.P: 150 °C; HR-MS: $[M]^+$ 376.0283 (Calcd. For $C_{17}H_{13}ClN_2O_4S$; 376.0292); IR (KBr): ν_{max} (cm^{-1}): 3047 (Ar—H stretching), 1536 (Ar C═C stretching), 1139 (C—O stretching), 704 (C—Cl stretching), 613 (C—S stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.91 (d, J = 8.8 Hz, 2H, H-2″ & 6″), 7.08 (s, 1H, H-5′), 6.98 (d, J = 8.8 Hz, 2H, H-3″ & 5″), 6.84 (s, 1H, H-2′), 5.94 (s, 2H, H-7′), 4.51 (s, 2H, H-8′), 3.85 (s, 3H, H-7″); ^{13}C NMR ($CDCl_3$, 100 MHz, δ/ppm): 164.6 (C-5), 163.8 (C-2), 158.2 (C-4″), 150.3 (C-4′), 149.7 (C-3′), 130.3 (C-1′), 125.7 (C-6′), 124.1 (C-1″), 117.4 (C-2″ & 6″), 116.7 (C-3″ & 5″), 110.8 (C-5′), 110.2 (C-2′), 101.6 (C-7′), 58.2 (C-7″), 32.4 (C-8′); EIMS (m/z): 378 $[M+2]^+$ (0.3%), 376 $[M]^+$ (1%), 341 (100%), 208 (16%), 200 (2%), 175 (7%), 169 (9%), 149 (2%), 139 (4%), 133 (6%), 134 (3%), 135 (4%), 107 (3%), 104 (9%), 51 (2%).

*2.5.9. 5-(2-(3,4-Methylenedioxyphenyl)ethenyl)-2-((6-chloro-3,4-methylenedioxy phenyl)methylthio)-1,3,4-Oxadiazole (**6i**)*

Gray amorphous solid; Yield: 98%; M.P: 116 °C; HR-MS: $[M]^+$ 416.0235 (Calcd. For $C_{19}H_{13}ClN_2O_5S$; 416.0255); IR (KBr): ν_{max} (cm^{-1}): 3063 (Ar-H stretching), 1654 (C═C stretching), 1543 (Ar C═C stretching), 1135 (C—O stretching), 701 (C—Cl stretching), 621 (C—S stretching); ^1H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.35 (d, J = 16.4 Hz, 1H, H-9″), 7.06 (s, 1H, H-5′), 7.02 (d, J = 1.6 Hz, 1H, H-2″), 6.98 (dd,

J = 8.0, 1.2 Hz, 1H, H-6″), 6.84 (s, 1H, H-2′), 6.81 (d, J = 8.0 Hz, 1H, H-5″), 6.79 (d, J = 16.4 Hz, 1H, H-8″), 5.99 (s, 2H, H-7″), 5.94 (s, 2H, H-7′), 4.50 (s, 2H, H-8′); ^{13}C NMR ($CDCl_3$, 100 MHz, δ/ppm): 165.3 (C-2), 164.8 (C-5), 150.8 (C-4″), 150.4 (C-4′), 149.3 (C-3′), 148.9 (C-3″), 140.5 (C-8″), 131.2 (C-1″), 130.7 (C-1′), 125.1 (C-6′), 121.3 (C-6″), 110.7 (C-5′), 110.3 (C-2′), 108.6 (C-9″), 107.8 (C-5″), 107.2 (C-2″), 101.7 (C-7′), 101.3 (C-7″), 32.4 (C-8′); EIMS (m/z): 418 $[M+2]^+$ (0.3%), 416 $[M]^+$ (1%), 381 (100%), 248 (8%), 215 (100%), 200 (7%), 189 (3%), 175 (2%), 173 (2%), 169 (7%), 147 (4%), 139 (4%), 134 (3%), 121 (7%), 104 (9%), 91 (13%), 51 (2%).

*2.5.10. 5-(4-Chlorophenoxymethyl)-2-((6-chloro-3,4-methylenedioxyphenyl)methyl thio)-1,3,4-Oxadiazole (**6j**)*

White amorphous solid; Yield: 98%; M.P: 96 °C; HR-MS: $[M]^+$ 409.9891 (Calcd. For $C_{17}H_{12}Cl_2N_2O_4S$; 409.9894); IR (KBr): ν_{max} (cm^{-1}): 3056 (Ar—H stretching), 1649 (C=C stretching), 1540 (Ar C=C stretching), 1131 (C—O stretching), 705 (C—Cl stretching), 617 (C—S stretching); ^{1}H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.25 (d, J = 8.8 Hz, 2H, H-3″ & 5″), 7.06 (s, 1H, H-2′), 6.92 (d, J = 8.8 Hz, 2H, H-2″ & 6″), 6.83 (s, 1H, H-5′), 5.95 (s, 2H, H-7′), 5.16 (s, 2H, H-7″), 4.49 (s, 2H, H-8′); ^{13}C NMR ($CDCl_3$, 100 MHz, δ/ppm): 165.6 (C-2), 164.4 (C-5), 153.7 (C-1″), 149.5 (C-4′), 148.1 (C-3′), 142.4 (C-4″), 130.8 (C-1′), 127.6 (C-3″ & 5″), 125.5 (C-6′), 113.6 (C-2″ & 6″), 110.9 (C-5′), 110.3 (C-2′), 101.8 (C-7′), 65.7 (C-7″), 32.4 (C-8′); EIMS (m/z): 414 $[M+4]^+$ (0.5%), 412 $[M+2]^+$ (1.2%), 410 $[M]^+$ (1.5%), 375 (100%), 242 (1%), 209 (1%), 200 (2%), 183 (2%), 169 (9%), 167 (6%), 141 (2%), 139 (2%), 134 (2%), 111 (15%), 104 (5%), 76 (63%), 51 (3%).

*2.5.11. 5-(Naphthalen-1-ylmethyl)-2-((6-chloro-3,4-methylenedioxyphenyl)methyl thio)-1,3,4-Oxadiazole (**6k**)*

White amorphous solid; Yield: 99%; M.P: 98 °C; HR-MS: $[M]^+$ 410.0496 (Calcd. For $C_{21}H_{15}ClN_2O_3S$; 410.0499); IR (KBr): ν_{max} (cm^{-1}): 3051 (Ar—H stretching), 1537 (Ar C=C stretching), 1126 (C—O stretching), 703 (C—Cl stretching), 613 (C—S stretching); ^{1}H NMR ($CDCl_3$, 400 MHz, δ/ppm): 8.09 (d, J = 8.4 Hz, 1H, H-4″), 7.86 (d, J = 8.0 Hz, 1H, H-8″), 7.81 (dd, J = 7.2, 2.0 Hz, 1H, H-5″), 7.54 (t, J = 7.2, Hz, 1H, H-7″), 7.51 (t, J = 8.0 Hz, 1H, H-6″), 7.44–7.41 (m, 2H, H-2″ & 3″), 6.93 (s, 1H, H-2′), 6.77 (s, 1H, H-5′), 5.92 (s, 2H, H-7′), 4.58 (s, 2H, H-11″), 4.37 (s, 2H, H-8′); ^{13}C NMR ($CDCl_3$, 100 MHz, δ/ppm): 165.9 (C-5), 164.6 (C-2), 149.9 (C-4′), 148.4 (C-3′), 141.7 (C-1″), 131.2 (C-5″), 130.6 (C-1′), 130.1 (C-4″), 129.7 (C-10″), 128.1 (C-7″), 127.4 (C-6″), 126.8 (C-3″), 125.3 (C-6′), 125.1 (C-2″), 123.9 (C-8″), 120.5 (C-9″), 110.8 (C-5′), 110.1 (C-2′), 101.4 (C-7′), 34.2 (C-11″), 32.7 (C-8′); EIMS (m/z): 412 $[M+2]^+$ (0.3%), 410 $[M]^+$ (1%), 375 (100%), 242 (4%), 209 (5%), 200 (2%), 183 (3%), 169 (9%), 167 (7%), 141 (4%), 139 (3%), 134 (1%), 127 (8%), 104 (2%), 101 (3%).

*2.5.12. 5-[1-(Phenylsulfonyl)piperidyl]-2-((6-chloro-3,4-methylenedioxyphenyl) methylthio)-1,3,4-oxadiazole (**6l**)*

White amorphous solid; Yield: 95%; M.P: 130 °C; HR-MS: $[M]^+$ 493.0537 (Calcd. For $C_{21}H_{20}ClN_3O_5S_2$; 493.0557); IR (KBr): ν_{max} (cm^{-1}): 3047 (Ar—H stretching), 1529 (Ar C=C stretching), 1407 (S=O stretching), 1123 (C—O stretching), 712 (C—Cl stretching), 615 (C—S stretching); ^{1}H NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.76 (d, J = 7.6 Hz, 2H, H-2‴ & 6‴), 7.59 (t, J = 7.6, 1H, H-4‴), 7.54 (t, J = 7.6 Hz, 2H, H-3‴ & 5‴), 7.00 (s, 1H, H-2′), 6.82 (s, 1H, H-5′), 5.94 (s, 2H, H-7′), 4.42 (s, 2H, H-8′), 3.70 (dt, J = 7.6, 4.0 Hz, 2H, H_{eq}-2″ & 6″), 2.82–2.79 (m, 1H, H-4″), 2.57 (dt, J = 6.8, 2.0 Hz, 2H, H_{ax}-2″ & 6″), 2.11 (dd, J = 13.5, 3.0 Hz, 2H, H_{eq}-3″ & 5″), 1.98 (dq, J = 11.0, 4.0 Hz, 2H, H_{ax}-3″ & 5″); ^{13}C NMR ($CDCl_3$, 100 MHz, δ/ppm): 170.7 (C-5), 164.7 (C-2), 149.9 (C-4′), 148.3 (C-3′), 144.8 (C-1‴), 133.5 (C-4‴), 130.4 (C-1′), 129.3 (C-3‴ & 5‴), 128.4 (C-2‴ & 6‴), 125.7 (C-6′), 110.7 (C-5′), 110.0 (C-2′), 101.5 (C-7′), 41.5 (C-2″ & 6″), 35.1 (C-4″), 32.7 (C-8′), 29.6 (C-3″ & 5″); EIMS (m/z): 495 $[M+2]^+$ (0.3%), 493 $[M]^+$ (1%), 458 (100%), 394 (1%), 325 (2%), 224 (7%), 200 (3%), 183 (3%), 169 (4%), 141 (9%), 139 (2%), 134 (2%), 104 (8%), 77 (10%), 51 (2%).

2.6. Antibacterial activity

The antibacterial activity of all the synthesized molecules was calculated by the reported method.[12–14]

2.7. Statistical analysis

Statistical analysis was accomplished by Microsoft Excel 2010 for all the measurements achieved in triplicate (threefold) and the results are presented as mean ± sem.

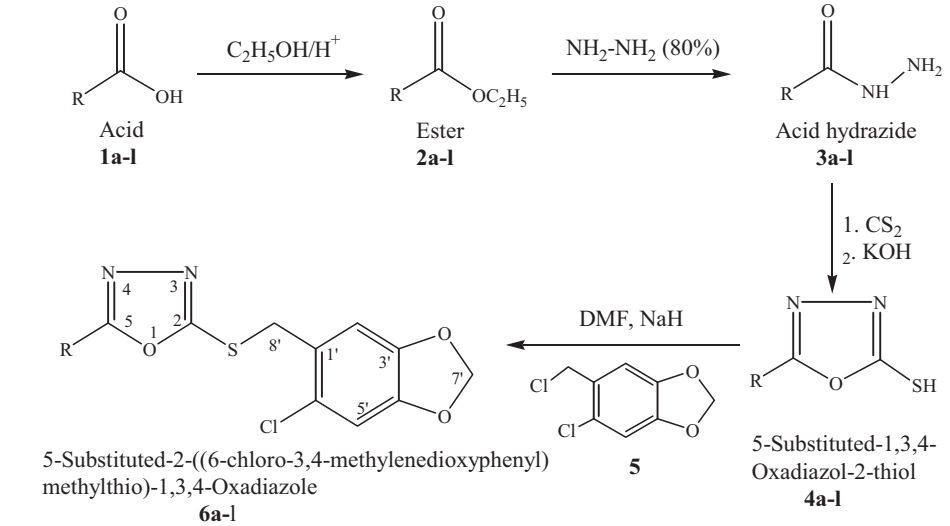
3. Results and discussion

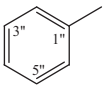
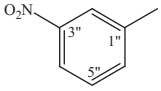
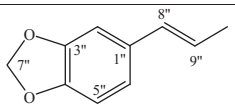
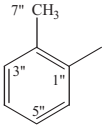
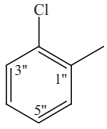
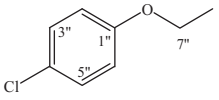
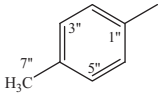
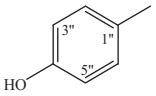
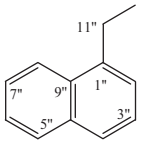
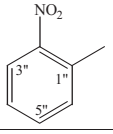
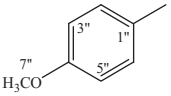
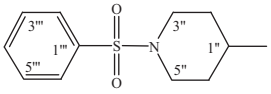
The *S*-substituted derivatives of 5-substituted-1,3,4-Oxadiazol-2-thiol have been synthesized by the protocol outlined in Scheme 1. The synthesized 5-substituted-2-((6-chloro-3,4-methylenedioxyphenyl)methylthio)-1,3,4-Oxadiazole derivatives, **6a–l**, were evaluated for antibacterial activity. The stepwise procedures, conditions and spectral characterization are explicated in experimental section.

3.1. Chemistry

We synthesized 5-substituted-2-((6-chloro-3,4-methylenedioxyphenyl)methylthio)-1,3,4-Oxadiazole derivatives in good to excellent yields with moderate antibacterial activities. These molecules were geared up by the stepwise synthesis involving esterification of aryl/aralkyl carboxylic acids (**1a–l**) into ethyl esters (**2a–l**) by refluxing with ethanol and small amount of sulfuric acid, nucleophilic substitution of **2a–l** by hydrazine into corresponding carbohydrazides (**3a–l**) by stirring, the intermolecular cyclization of **3a–l** to 5-substituted-1,3,4-Oxadiazol-2-thiols (**4a–l**) by refluxing with CS_2/KOH in ethanol and finally the electrophilic substitution of **4a–l** by 6-chloro-3,4-methylenedioxybenzyl chloride (**5**) in DMF and NaH (Scheme 1). The purported structures of all the molecules were confirmed by the spectral data, elaborated in experimental section.

White amorphous solid, compound **6a**, was synthesized in a good yield of 99% with 155 °C melting point. Molecular formula, $C_{16}H_{11}ClN_2O_3S$ was checked out by molecular ion peak at m/z 346 & $[M+2]^+$ ion peak at m/z 348 in EI-MS and by integration curve of protons in ^{1}H NMR spectrum. The IR spectrum supported the structure by different absorption



Comp.	R	Comp.	R	Comp.	R
6a		6e		6i	
6b		6f		6j	
6c		6g		6k	
6d		6h		6l	

Scheme 1 Synthesis of 5-substituted-2-((6-chloro-3,4-methylenedioxyphenyl)methylthio)-1,3,4-Oxadiazole.

bands, mainly at 1603–1630 cm^{-1} for C═N (stretching) and at 1051–1079 cm^{-1} for C—O (stretching) in all the molecules affirming the 1,3,4-Oxadiazole ring. In the EI-MS, a base peak was noted at *m*/*z* 311 after the removal of chlorine radical from the molecule. The prominent peaks appeared at *m*/*z* 178 for 5-phenyl-1,3,4-Oxadiazol-2-thiol cation and at *m*/*z* 169 for 6-chloro-3,4-methylenedioxyphenyl)methyl cation. For more details, the mass fragmentation pattern of 5-phenyl-2-((6-chloro-3,4-methylenedioxyphenyl)methylthio)-1,3,4-Oxadiazole (**6a**) was sketched in Fig. 1. The ^{1}H NMR spectrum indicated four signals, for the 6-chloro-3,4-methylenedioxybenzyl group attached at *S*-position of the 1,3,4-Oxadiazole group, at δ 7.09 (s, 1H, H-2′) and 6.84 (s, 1H, H-5′) for aromatic protons and at δ 5.94 (s, 2H, H-7′) and 4.53 (s, 2H, H-8′) for aliphatic protons. The five protons of the phenyl group were attributed by one doublet of doublet at δ 7.98 (dd, J = 8.0, 1.2 Hz, 2H, H-2″ & 6″) and one multiplet in the range of 7.50–7.45 (m, 3H, H-3″ to 5″). The ^{13}C NMR spectrum (BB and DEPT) showed fourteen signals resonating for seven quaternary, seven methylene and two methine carbons. The order for seven signal quaternary carbons was δ (ppm) 165.4 (C-5), 164.6 (C-2), 150.3 (C-4′), 148.7 (C-3′), 129.8 (C-1″), 129.5 (C-1′) and 125.7 (C-6′). The seven methine carbons were nominated by five signals at δ (ppm) 135.2 (C-3″ & 5″), 132.8 (C-2″ & 6″), 130.4 (C-4″), 110.9 (C-5′) and 110.2 (C-2′). The two signals at δ (ppm) 101.9 (C-7′) and 32.7 (C-8′) were assigned to two methylene groups of benzodioxane moiety. All this accumulative discussion, corroborated the structure of **6a** as 5-phenyl-2-((6-chloro-3,4-methylenedioxyphenyl)methyl thio)-1,3,4-Oxadiazole. The structures of other synthesized compounds were confirmed on the basis of spectral data, described in experimental section.

3.2. Antibacterial activity (in vitro)

The %age inhibition (percentage inhibition) and MIC (minimum inhibitory concentration) results of *in vitro* antibacterial activity of the synthesized molecules against bacterial strains of Gram-bacteria are depicted in Table 1 and Table 2, respectively.

The screening of the synthesized series as 5-substituted-2-((6-chloro-3,4-methylenedioxyphenyl)methylthio)-1,3,4-Oxadi-

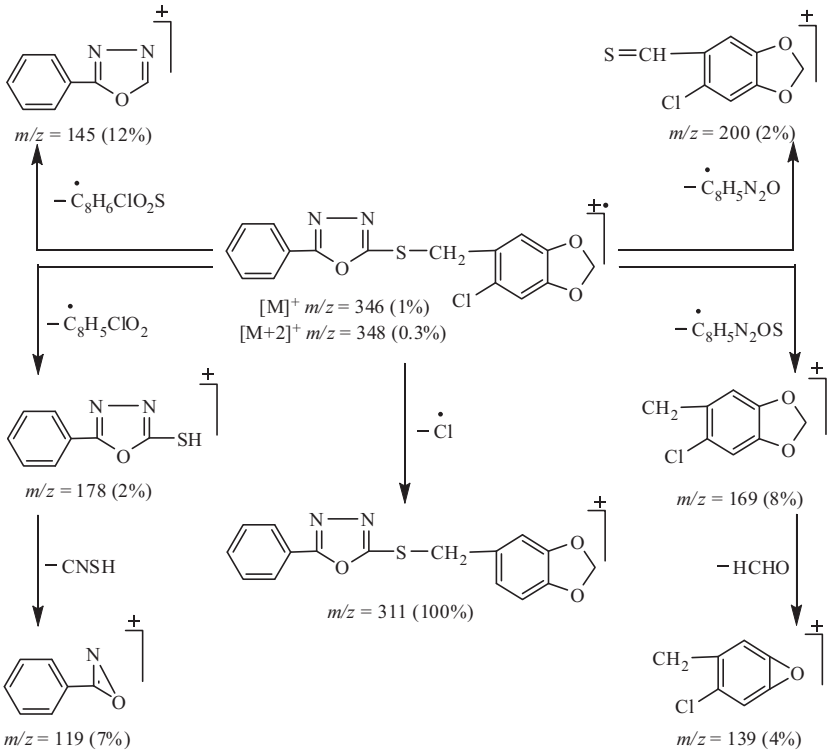


Figure 1 Mass fragmentation pattern of 5-phenyl-2-((6-chloro-3,4-methylenedioxyphenyl)methylthio)-1,3,4-Oxadiazole (**6a**).

Table 1 % age inhibition values of antibacterial activity.

Compound	*S. typhi (−)*	*E. coli (−)*	*P. aeroginosa (−)*	*B. subtilis (+)*	*S. aureus (+)*
%age inhibition					
6a	24.52 ± 4.52	64.44 ± 3.11	47.83 ± 4.33	38.85 ± 1.38	40.05 ± 0.14
6b	34.04 ± 1.35	56.67 ± 1.89	50.33 ± 3.25	48.85 ± 3.23	45.05 ± 0.95
6c	47.88 ± 2.98	62.94 ± 2.39	59.75 ± 3.83	53.35 ± 4.04	54.41 ± 0.68
6d	49.23 ± 4.81	53.44 ± 1.11	58.58 ± 1.33	55.15 ± 4.31	56.59 ± 5.00
6e	52.31 ± 3.65	64.83 ± 5.00	59.96 ± 0.21	41.12 ± 2.88	58.09 ± 2.09
6f	54.47 ± 4.47	72.28 ± 1.28	61.17 ± 4.25	58.04 ± 4.96	50.68 ± 0.23
6g	52.60 ± 0.00	61.33 ± 3.00	53.83 ± 1.58	61.31 ± 2.46	41.41 ± 0.86
6h	58.41 ± 4.57	70.33 ± 5.00	60.80 ± 4.79	56.54 ± 3.00	45.77 ± 1.23
6i	39.86 ± 1.39	57.94 ± 1.94	46.96 ± 0.13	40.54 ± 1.08	43.50 ± 3.50
6j	60.38 ± 4.33	74.11 ± 5.00	66.50 ± 2.25	58.46 ± 1.00	65.05 ± 3.86
6k	54.47 ± 3.99	57.67 ± 1.44	63.29 ± 0.46	53.73 ± 0.42	54.86 ± 2.05
6l	56.59 ± 1.88	70.94 ± 0.72	61.04 ± 0.21	54.04 ± 3.27	37.23 ± 3.32
Ciprofloxacin	90.76 ± 0.79	92.02 ± 1.97	90.32 ± 1.09	89.65 ± 2.00	91.22 ± 2.32

azole against Gram-negative and Gram-positive bacteria displayed that most of them executed moderate potential. The molecules, **6f**, **6j** and **6k** showed moderate activity against all the bacterial strains. *E. coli* and *P. aeroginosa* (except **6a** and **6i**) were inhibited by all the molecules of this series. A few molecules remained inactive such as, **6c** & **6d** against *Salmonella typhi*; **6e** against *Bacillus subtilis*; and **6g**, **6h** & **6l** against *Staphylococcus aureus*. The most active molecule among the whole series was **6j** which exhibited good inhibitory action against all the strains. The MIC values of this molecule have been noted as 14.90 ± 2.41, 11.37 ± 4.23, 11.40 ± 2.88, 12.81 ± 2.44 and 13.68 ± 3.21 μM relative to ciprofloxacin with MIC values of 8.33 ± 1.21, 8.94 ± 1.87, 8.14 ± 1.32, 9.04 ± 2.01 and 8.98 ± 1.44 μM. The prominent activity of this molecule owes to the presence of the 4-chlorophenoxy group in the molecule.

4. Conclusion

The presented series of compounds was synthesized in good yields to inaugurate some new antibacterial agents. Among the synthesized molecules, all the molecules have exhibited moderate activity against *E. coli* and the molecule, **6j**, was

Table 2 MIC values of antibacterial activity.

MIC					
Compound	*S. typhi (−)*	*E. coli (−)*	*P. aeroginosa (−)*	*B. subtilis (+)*	*S. aureus (+)*
6a	–	13.97 ± 3.85	–	–	–
6b	–	16.17 ± 5.00	19.11 ± 1.30	–	–
6c	–	15.73 ± 1.54	14.90 ± 1.14	16.76 ± 3.38	17.75 ± 4.12
6d	–	17.75 ± 3.08	12.52 ± 1.20	16.65 ± 1.23	15.84 ± 4.00
6e	18.03 ± 3.63	13.43 ± 5.00	15.01 ± 1.55	–	16.73 ± 3.13
6f	17.37 ± 1.87	11.73 ± 5.00	14.74 ± 4.35	17.02 ± 3.38	19.03 ± 2.98
6g	18.92 ± 4.12	14.51 ± 5.00	17.54 ± 3.45	15.54 ± 1.81	–
6h	15.00 ± 2.43	11.48 ± 2.46	15.08 ± 1.95	16.89 ± 3.50	–
6i	–	14.87 ± 3.08	–	–	–
6j	14.90 ± 2.14	11.37 ± 4.23	11.40 ± 2.86	12.81 ± 2.44	13.68 ± 3.21
6k	17.28 ± 3.25	15.67 ± 3.00	15.56 ± 3.55	16.99 ± 4.19	17.59 ± 3.14
6l	16.27 ± 3.22	13.19 ± 3.31	14.51 ± 4.45	18.30 ± 1.25	–
Ciprofloxacin	8.33 ± 1.21	8.94 ± 1.87	8.14 ± 1.32	9.04 ± 2.01	8.98 ± 1.44

Note: Minimum inhibitory concentration (MIC) was measured with suitable dilutions (5–30 μg/ well) and results were calculated using EZ-Fit Perrella Scientific Inc. Amherst USA software.

the most active among this series. The synthesized molecules might be helpful for the pharmaceutical industries in drug discovery program.

5. Conflict of interest

None declared.

Acknowledgement

The authors pay much thanks to the Higher Education Commission (HEC) of Pakistan for financial support concerning research work and spectral characterization.

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